

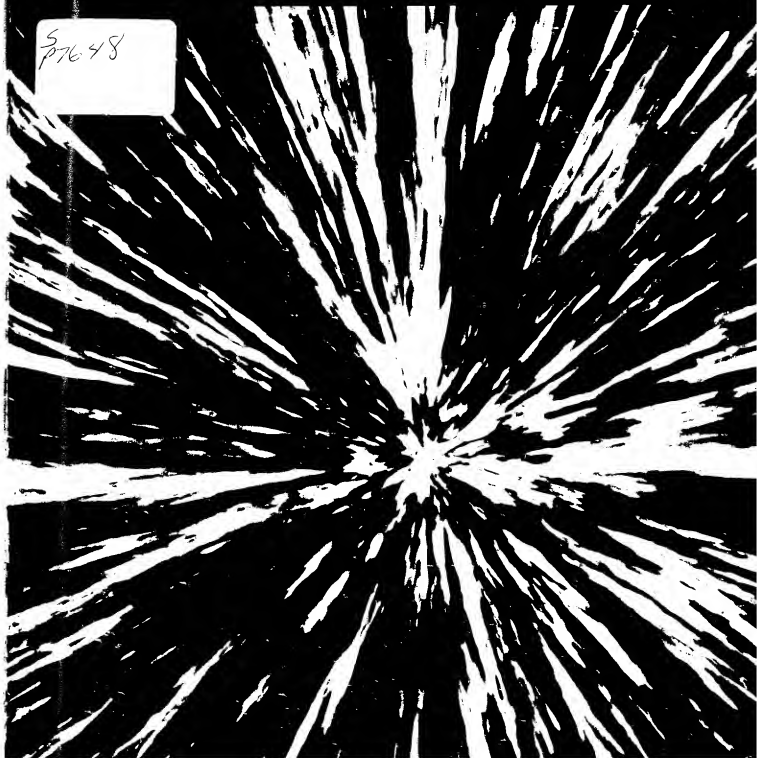
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Biodegradable brush-like graft polymers from poly(D,L-lactide) or poly(D,L-lactide-co-glycolide) and charge-modified, hydrophilic dextrans as backbone – *in-vitro* degradation and controlled releases of hydrophilic macromolecules

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Parenteral delivery systems for proteins and peptides based on aliphatic polyesters are currently the subject of intensive research efforts. Linear polyesters of lactic acid and glycolic acid present significant problems with respect to the modulation of release properties of higher molecular weight substances, such as proteins. The molecular architecture of poly(lactic acid) (PLA) and its copolymers with glycolic acid (PLG), can be modified by the introduction of a hydrophilic charge-containing backbone, e.g. dextran sulfate sodium (DSS) or diethylaminoethyl dextran chloride (DEAED). The resulting branched molecular structure should offer additional possibilities to manipulate the degradation and release properties of parenteral delivery systems. The degradation of the graft PLG is accelerated significantly by the nonlinear structure, which contains many short biodegradable branches attached to a hydrophilic backbone molecule. The mechanism of the polymer degradation is influenced by the backbone substances. In the case of DEAED as backbone, the predominant chain scission of the graft polyester occurs in a random hydrolytic ester cleavage, similar to PLG. By contrast, a nonrandom chain scission in the vicinity of the branching points of the backbone was found for DSS-PLG. The erosion of the graft PLG proceeds more rapidly in the centre of the devices than at the surface. In contrast to linear PLG, the release of FITC-dextran and bovine serum albumin (BSA) from the microspheres prepared from the graft PLG is continuous. Graft PLGs offer additional possibilities for adjusting the release of proteins and peptides from biodegradable parenteral delivery systems. © 1998 Published by Elsevier Science Ltd. All rights reserved.

(Keywords: brush-like graft polyesters; *in-vitro* degradation; morphology)

INTRODUCTION

For the past few decades, biodegradable polyesters, e.g. polylactide (PLA) and its copolymer with glycolide (PLG), have been widely utilized for controlled drug delivery systems. These biodegradable and biocompatible polyesters have shown an excellent safety record¹. Recently, these polyesters have been used to control protein and peptide release from parenteral depot delivery systems^{2–5}.

To successfully deliver protein drugs for a desired period, it is essential to understand the degradation properties of the polymer as well as the stability of the protein in the polymeric matrix. A well-proven advantage of PLG is the versatility of modifying polymer properties and performance characteristics by copolymerization. For application in controlled drug delivery, it is necessary to adjust drug release and polymer degradation rates by careful manipulation of monomer stereochemistry, comonomer ratio, and polymer molecular weight⁶. The degradation properties of PLA and PLG depend on many factors, e.g. molecular weight, copolymer composition and crystallinity. In general,

a bulk erosion mechanism has been considered as the main degradation pathway for PLA and PLG. In this case a random scission of ester bonds in the polymer chain proceeds homogeneously throughout the device^{7–9}. Recently, it was reported that massive devices, such as implants of PLA and PLG degrade via a heterogeneous mechanism^{10–12}, whereby the degradation proceeds more rapidly in the centre of the implant than at the surface. This is attributed to the autocatalytic action of the carboxylic acid end group of the degradation products which are generated and retained in the matrix.

The drug release from PLA and PLG is, therefore, controlled by both drug diffusion and polymer erosion. Despite considerable efforts, drug release from PLG devices is often not satisfactorily controlled. Release patterns deviating from an ideal 'infusion-like' profile are not uncommon and especially drug candidates with higher molecular weights, e.g. proteins, have shown *in vivo* plasma-level/time curves with clear discontinuities, often designated as 'polyphasic'¹² or 'triphasic'¹³.

The molecular architecture of linear PLG can be modified by introducing hydrophilic and charge-modified carbohydrates to synthesize graft polymers. These branched polyesters offer a potential to improve the 'polyphasic' or 'triphasic' release profiles^{14,15}. Graft PLGs were used in

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controlled bromocriptine release from microspheres, yielding a continuous drug release profile over one month¹⁴.

In a study previously reported¹⁵, the synthesis and characteristics of the graft PLA and PLG containing hydrophilic charge-modified dextrans (DSS and DEAED) as backbones was communicated. Evidence for the branched structure of the graft PLG is based on various experimental techniques such as laser light scattering, NMR, and intrinsic viscosity measurements. Preliminary data on the *in-vitro* degradation of the graft PLA and PLG show accelerated degradation rates and erosion of the matrix, compared to linear PLG. In this paper, we investigate the degradation mechanism of these graft polymers, their morphology during degradation, and the controlled release properties of microspheres containing hydrophilic macromolecules, e.g. FITC-dextran and bovine serum albumin (BSA).

EXPERIMENTAL

Materials

Graft poly(lactide-co-glycolide)s containing dextran and charge-modified dextrans bearing a net negative charge (dextran sulfate sodium, DSS, Sigma) or a positive charge (diethylaminoethyl dextran chloride, DEAED, Sigma) were synthesized as described previously¹⁵. Fluorescein isothiocyanate labelled dextran (FITC-dextran, M_w 40 000) was prepared from dextran (M_w 40 000, Sigma) and bovine serum albumin (BSA fraction V, M_w 67 000, Merck) was used as received.

Preparation of films

Films were cast from 5% methylene chloride solution (w/v) on Teflon coated plates. Residual solvents were removed *in vacuo* at room temperature for 2 days until constant weight was obtained, and subsequently cut into $50 \times 5 \text{ mm}^2$ specimens. The film thickness was found to be $200 \pm 20 \mu\text{m}$.

Preparation of microspheres

Microspheres were prepared using a W/O/W double emulsion method as described in our previous paper¹⁶. The loading of model compounds was assayed photometrically at different wavelengths: 493 nm (FITC-Dextran), 278 nm (BSA) using a Shimadzu UV-160 spectrophotometer. The sizes of the microspheres were analysed by laser light diffraction using a Mavern Mastersizer MSX in an aqueous dispersion containing Tween 80 (0.5%).

In-vitro degradation

Films of the polymers immersed in 50 ml of 0.2 M phosphate buffer saline (pH 7.2) were stirred in a rotating metal-block thermostat (Rotatherm, Liebisch, Germany) at 15 rpm and 37°C. At preset intervals, the samples were recovered, and frozen at -20°C for 2 h then freeze dried *in vacuo* (0.4 mbar) at -20°C for 48 h followed by secondary drying at room temperature for 48 h. Molecular weights (GPC), mass loss (gravimetry), film morphologies (SEM) and thermal properties (DSC) were analysed. The surface of the film was eluted by 2 ml of CH_2Cl_2 at a flow rate of 0.1 ml/sec. The solution was collected and concentrated to 0.5 ml for GPC measurement.

Water-uptake

A dry film specimen was immersed in phosphated buffer saline (pH 7.2) and recovered after 24 h, the weight of the

wet film was determined after the water on the surface was removed by blotting, the water content was calculated from the weight increase of the films.

In-vitro drug release

All *in-vitro* release studies were carried out in phosphate buffered saline at pH 7.2. Weighed amounts of microspheres (ca. 20 mg) were placed in 5 ml dissolution medium in 20 ml screw cap glass test tubes ($n = 3$) and rotated at 37°C in a metal-block thermostat at 30 rpm. At preset time intervals, the vials were centrifuged at 5000 rpm for 10 min, and then 3 ml of clear solution were drawn and replaced by fresh dissolution medium. The drug concentrations in the supernatant were determined spectrometrically.

Scanning electron microscopy (SEM)

Films were fixed on aluminium studs and coated with gold using a sputter coater S 150 from Edwards GmbH. The samples were sputter coated three times (2 min) under vacuum (0.1 mmHg) in an argon atmosphere at a current intensity of 20 mA. The morphologies of the films were then analysed by scanning electron microscopy, SEM (Model 501 S, Hitachi, Tokyo, Japan).

Analytical methods

GPC was carried out on Merck size exclusion columns (Lichrogel PS mix and Lichrogel PS 40, 10 μm), thermostated at 25°C, using methylene chloride as solvent and a differential refractometer as detector (Merck RI 71). Molecular weights were calculated by the universal calibration method using polystyrene reference materials (Merck, M_w 3250; 5100; 19 600; 34 000; and 87 000). DSC measurement was carried out using a differential scanning calorimeter (Perkin Elmer DSC 7) in sealed aluminium pans under nitrogen atmosphere. Thermograms covering a range of -20 to 200°C were recorded at heating and cooling rates of $10^\circ\text{C}/\text{min}$.

RESULTS AND DISCUSSION

In-vitro degradation of the graft PLA and PLG

The modification of the chemical structure of polymer may lead to improvements in both physico-chemical and drug release properties. Graft PLG have not only a different architecture compared to the linear PLG, but also different chemical and physical properties. In our previous report¹⁵, we investigated the biodegradability of the graft PLA and PLG containing DSS and DEAED as backbones. We found significantly accelerated *in-vitro* degradation rates of the film prepared from these graft polyesters. In extension to these studies, the kinetics of the hydrolytic cleavage and the changes in the morphology of the graft PLG are reported here.

The composition and molecular weights of the graft PLG used in this investigation are summarized in Table 1. The branched polyesters were synthesized by grafting PLG onto the charge hydrophilic backbone as reported earlier¹⁵.

Molecular weights and polydispersities

In Figure 1 the degradation properties of the graft polyesters with diethylaminoethyl dextran chloride as backbone (DEAED-PLA and DEAED-PLG) are shown. As expected, the number-average molecular weights (M_n) decrease rapidly, accompanied by an increase in the

polydispersity up to 8–12. The decrease in weight-average molecular weights (M_w) proceeds more slowly. The change in the GPC traces of DEAE-PLG (63/37) as a function of time is given in Figure 2A. After incubation for one week in buffer, a shift of the molecular weight was observed leading to polydispersities. The composition and initial molecular weight have a significant influence on the degradation behaviour. Higher LA content and higher initial molecular weight lead to a slower degradation.

The degradation of biodegradable polyesters in water can occur by random or nonrandom chain scission. The predominant mechanism of hydrolysis of linear PLA and PLG is usually considered to proceed as random cleavage of ester bonds. The terminal carboxylic groups have only a limited influence on the rate of hydrolysis in this particular model^{7–9}. Experimental and theoretical descriptions of the random chain scission of linear polymers has been studied for many years by the application of the analytical

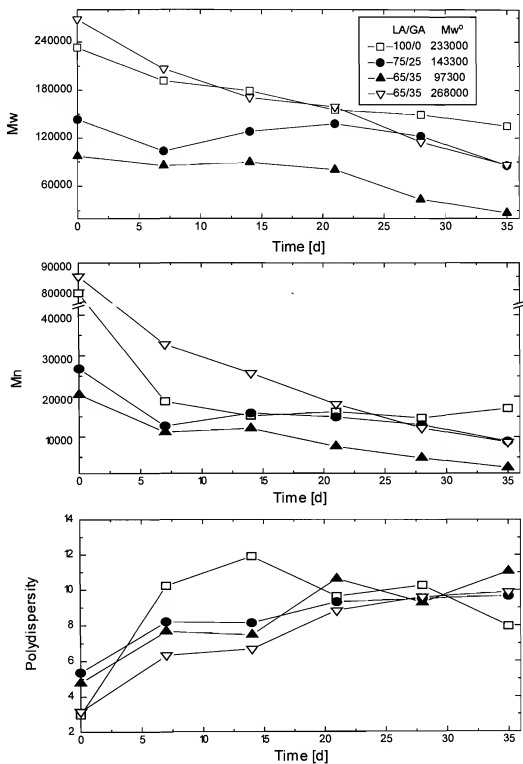


Figure 1 In-vitro degradation of DEAE-PLGs in phosphate buffer saline (pH 7.2) at 37°C—changes in molecular weights and polydispersities

techniques¹⁷⁻²⁴ for the limited initial molecular weight distribution and by Monte Carlo simulations²⁵⁻²⁸. For the degradation reactions of linear polymers, even nonrandom degradation has already been studied²⁹⁻³¹, but the publications concerning the description of the degradation of branched or grafted polymeric molecules are scarce. Tobita reported recently the random scission of the star and branched polymers^{32,33}. Using Monte Carlo simulation, he demonstrated that the random scission of branched polymer yields different results regarding the molecular weight and polydispersity than linear polymers. The decrease of the weight-average molecular weight of the branched polymer was slower than that of the linear polymer, accompanied by a broader polydispersity. Our findings are in line with the theoretical model proposed by Tobita^{32,33}, suggesting a random hydrolytic kinetics in the degradation of DEAED-PLG.

The degradation behaviour of DSS-PLG is shown in Figure 3 for different compositions, demonstrating some differences compared to the positively-charged DEAED-PLG. In this case a fast decrease in M_w was observed as a function of incubation time. The increase in the polydispersity was less pronounced and GPC traces indicated that the degradation of the DSS-PLG was not as heterogeneous as in the case of DEAED-PLG (Figure 2B). Interestingly, the molecular weights have only a limited

Table 1 Chemical and physical properties of the polymers used in study

No.	Backbone LA/GA	M_n^a	M_w^a	D^a	T_g °C	
1	DEAED 100/0	233 000	78 700	2.96	52	
2	DEAED 75/25	143 000	26 800	5.35	40	
3	DEAED 65/35	268 000	87 800	3.05	39	
4	DEAED 63/37	93 700	20 400	4.78	38	
5	DEAED 50/50	43 000	15 300	2.81	37	
6	DSS 75/25	57 000	21 600	2.63	—	
7	DSS 62/38	124 000	87 400	1.42	34	
8	DSS 60/40	50 900	23 300	2.19	34	
9	DSS 50/50	54 000	25 100	2.15	33	
10	dextran	50/50	43 000	15 300	2.81	37
10	—	50/50	42 000	15 000	2.80	44

^aDetermined by GPC

influence on the degradation rate of DSS-PLG, when we compared the batches 62/38 and 60/40 (Figure 3). After 1 week of incubation in phosphate buffered saline, the molecular weights M_n reached 10 000 g/mol in both cases.

Due to the branched structure of DSS- and DEAED-PLG, the hydrolytic degradation behaviour differs significantly from that of linear PLG, and we observed faster degradation rates for DSS- and DEAED-PLG¹⁵. Since the backbones of DEAED-PLG and DSS-PLG are hydrolytically stable, cleavage of the polymer chains occurs only in the branches, yielding short linear fragments. The backbones become

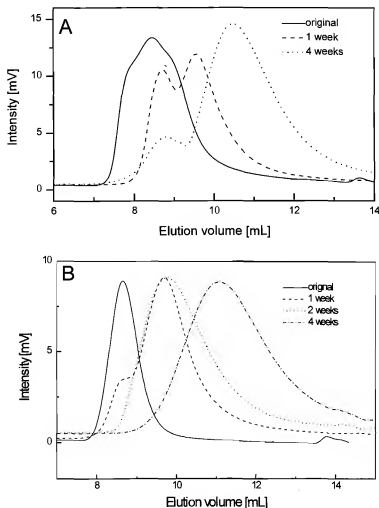


Figure 2 GPC traces of the original and degraded graft PLGs. A, DEAED-PLG(63/37); B, DSS-PLG(62/38)

water soluble when most of the branches have been cleaved by hydrolysis. The experimental results for DEAED-PLG suggest that the degradation occurs by random scission mechanism as proposed by Tobita^{32,33}. Random scission of the polymer chains is limited to the branches as schematically shown in Figure 4, yielding a mixture of DEAED-PLG and PLG, accompanied by a rapid increase in the polydispersity and a slow decrease in M_w as a function of time. The position of chain hydrolysis is not influenced significantly by the backbone and the accelerated degradation is mainly due to the architecture of the chain affecting the glass transition temperature¹⁵.

In contrast, the chain scissions of DSS-PLG may take place in the vicinities of the connection points of the branches to the backbones, yielding the hydrophilic negatively-charged dextran sulfate which will not be detected in GPC due to its insolubility in CH_2Cl_2 . In the case of DSS-PLG, the predominant chain scission reaction is most likely nonrandom in nature and occurs at the connection point between backbone and PLG branch. Therefore, the negatively charged backbone (DSS) may affect the cleavage reaction in a catalytic manner, accelerating the hydrolysis of the ester bond in the vicinity of the backbone, as schematically shown in Figure 4.

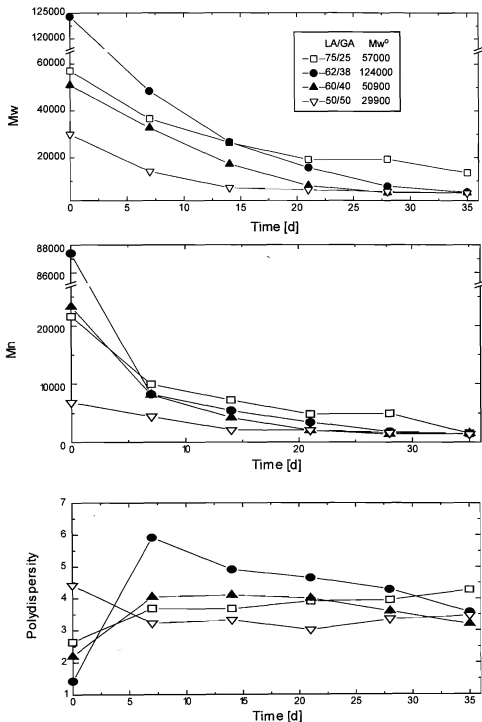


Figure 3 In-vitro degradation of DSS-PLGs in phosphate buffer saline (pH 7.2) at 37°C—changes in molecular weights and polydispersities

Glass transition temperature

Thermochemical properties are also important for the degradation of the polymer. In general, a polymer with a higher glass transition temperature (T_g) or a higher crystallinity will be less degradable. Graft PLGs have steric chain architectures, which decrease the interactions of molecules and therefore T_g and crystallinity of DSS-PLG and DEAED-PLG will also be reduced compared to PLG¹⁵. The permeation of water can also influence the thermal properties. Using differential scanning calorimetry (DSC) analysis, the T_g s of the degraded polymers in dry or wet state were determined (Figure 5). In the wet state, water acts in the polymeric matrix as a plasticizer, lowering the T_g s of graft PLG rapidly as a function of the incubation with buffer medium. After one day incubation the T_g for both graft PLGs are lower than the incubation or body temperature which means that the devices will be not in the glassy state. In the dry state, the T_g of DEAED-PLG remained unchanged for 3 weeks, whereas the T_g of DSS-PLG decreased after 1 week. Because of the random chain scission mechanism, higher molecular weights of DEAED-PLG are maintained during the degradation affecting the thermal properties of these polymers, but not as dramatically as in the case of DSS-PLG.

Morphologies of the films

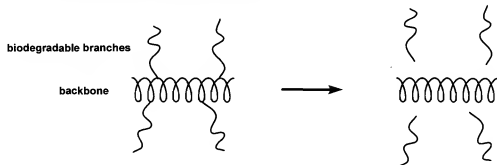
The structural changes of films prepared from DSS-PLG and DEAED-PLG regarding the morphology of their surface and matrix during the degradation were analysed by scanning electron microscopy (SEM). SEM allows the visualization of pore-formation. While linear PLG is thought to degrade in a heterogeneous fashion from

'inside-out', leading to pore formation inside the polymeric matrix and hollow structures³⁴, a different degradation behaviour was found for the graft PLG where a substantial mass loss was observed at the early degradation period¹⁵.

After 5 weeks incubation in phosphate buffered saline at 37°C, films of DEAED-PLG(63/37) showed a nearly unchanged structure of the surface in SEM micrographs (Figure 6A,B), characterized by a smooth and dense structure. In cross-section of the same film specimen, the SEM detected significant porosity (Figure 6C), which is attributed to a heterogeneous degradation process. It is interesting to note that the thickness of the surface layer was ca. 10 μm . This smooth surface layer was isolated by an extraction with dichloromethane to determine the molecular weight by GPC. The ratio of molecular weights in the surface layer and in the matrix are plotted as a function of incubation time (Figure 7), both DEAED-PLG(63/37) and DSS-PLG(75/25) (Figure 6D,E,F) showed a pronounced polarity between matrix and surface, indicative of the degradation mechanism of these graft PLG occurring from 'inside-out', similarly to linear PLG^{10-12,34}. The surface layer remained smooth for up to 8 weeks, and then became rough and porous. The higher hydrophobicity, either due to the weight-average molecular weight of DEAED-PLG or due to the monomer ratio of 75/25 in the case of DSS-PLG(75/25) leads to a slower random scission of the ester bonds during degradation. The diffusion of the oligomers is reduced, leading to a change of the bulk pH and an accelerated degradation in the matrix³⁵.

In the case of the DSS-PLG(62/38) the surface layer deteriorated after 3 weeks and became rough and porous (data not shown). The pores in the bulk were larger than on

Nonrandom hydrolysis



Random hydrolysis:

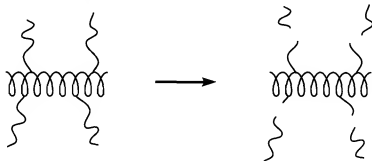


Figure 4 Schematic diagram of the hydrolytic mechanism of the graft PLGs

the surface and also larger than in the bulk of DEAED-PLG. This open porous structure with a pore gradient, with smaller pores close to the surface of the film, suggests a heterogeneous hydrolytic rate in the polymeric matrix. Compared to DEAED-PLG, the difference in the molecular weights of the surface and bulk of the film was much smaller than that of DEAED-PLG(63/37) as shown in Figure 7. The nonrandom chain scission leads to a fast decrease in the molecular weight (either M_w or M_n) and the thermal properties. The diffusion of acidic degradation products is

higher than in DEAED-PLG. This increased permeability of the film is reflected in the water uptake during degradation as shown in Figure 8. Faster degradation of DSS-PLG(62/38) leads to a more rapid water uptake of the matrix, promoting the diffusion of the oligomers from the matrix. The ratio of lactic acid, LA, and glycolic acid, GA, in the branches of the graft PLG also affects the degradation mechanism. At molar ratios of LA/GA = 50/50, the fastest rates of degradation were observed, similar to PLG¹. In this case a more homogeneous degradation of the polymeric

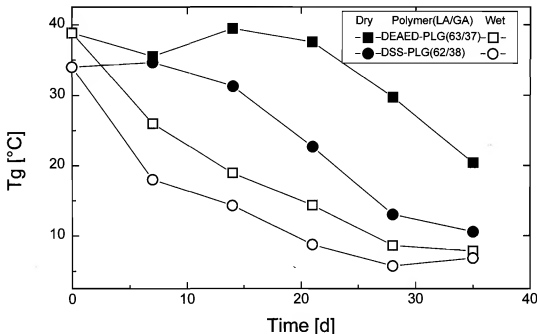


Figure 5 Glass transition temperatures of the degraded films of the graft PLGs with incubation time

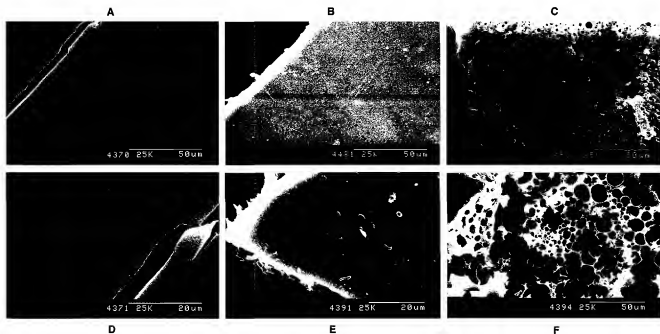


Figure 6 Morphologies of DEAED-PLG(63/37) and DSS-PLG(75/25) during *in-vitro* degradation

matrix of DSS-PLG(50/50) and DEAED-PLG(50/50) is obtained. After 3 weeks for both graft PLGs the rough and porous state of the surface and bulk of the film was evidenced by SEM as shown in Figure 9. The water content increased proportionally to the LA/GA ratio (Figure 8). There is no significant difference between molecular weight at the surface and in the bulk of the films from the graft PLG (Figure 7). These observations suggest that the physical properties of the polymeric matrix play an important role in the hydrolytic degradation mechanism of the matrix. By manipulating the composition and structure of graft PLG the well known heterogeneous degradation mechanism of linear PLG³⁴ can be rendered homogeneous. This is an important aspect for parental delivery systems containing peptides and proteins¹⁶.

Release of hydrophilic macromolecules from microspheres prepared from graft PLG

Graft PLG of different compositions in the backbone and a molar ratio of LA/GA = 50/50 were used to prepare biodegradable microspheres containing hydrophilic macromolecules as marker substances, such as fluorescently labelled FITC-dextran and BSA. A modified W/O/W double emulsion method was used to prepare the microspheres^{36,37}. The microencapsulation using graft PLG with a theoretical loading of marker substances of 5% (w/w) did not pose any problem. As shown in Table 2, the encapsulation efficiency was usually in the range of 85–95% and yields were more than 90%. The microspheres from graft PLG show nonporous and smooth surfaces as determined by SEM and the average size was found to be

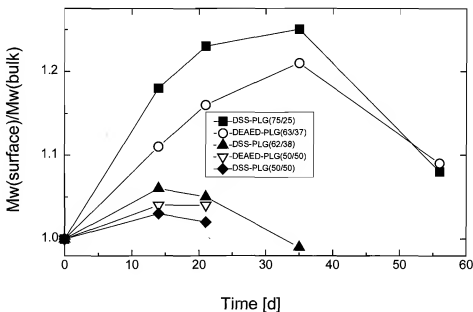


Figure 7 Weight-average molecular weight of the degraded graft PLG film-surface and bulk

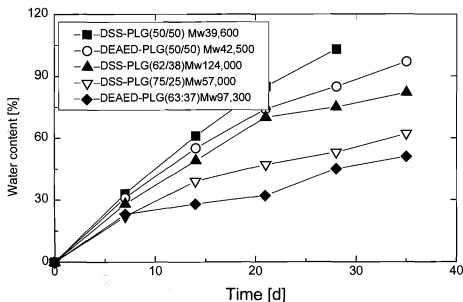


Figure 8 Water content in the polymeric matrix

30–60 μm which was preferred for intramuscular or subcutaneous injections.

FITC-dextran (M_w 40 000) is a fluorescent labelled, hydrophilic polyglycan. This marker substance is relatively stable under *in vitro* and *in vivo* conditions¹⁶. It was frequently used as a model compound for proteins. The release of FITC-dextran from the microspheres prepared from linear PLG showed a triphasic release pattern. After an initial burst, the *in vitro* release rate decreased to low values and recommenced when the erosion of PLG became noticeable, leading to a characteristic sigmoidal release profile⁶. The *in vitro* profiles of FITC-dextran from the graft PLG(50/50) were improved significantly (Figure 10). After a small initial burst, almost continuous release rates

were obtained for both DSS-PLG(50/50) and DEAED-PLG(50/50). The effect of charged groups on the backbone significantly influences the *in vitro* release of FITC-dextran from microspheres. The uncharged dextran-PLG shows similar *in vitro* release characteristics as the linear PLG up to the 20th day. Then an accelerated release is seen, probably due to the degradation of the microspheres. The more rapid degradation of the graft PLG, especially DSS-PLG(50/50) and DEAED-PLG(50/50), leads to changes in the morphology of the microspheres similar to those described above. The formation of a porous, water-filled structure facilitates both the diffusion and release of FITC-dextran as well as the degradation of the microspheres, leading to a continuous release of the marker molecule.

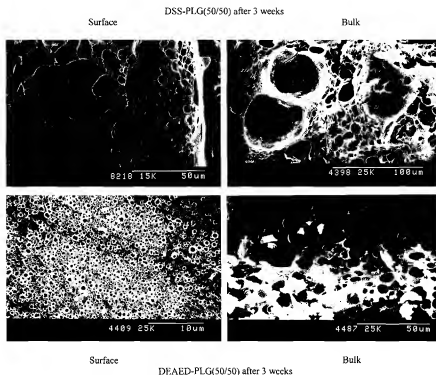


Figure 9 Morphologies of DSS-PLG(50/50) and DEAED-PLG(50/50) during *in vitro* degradation

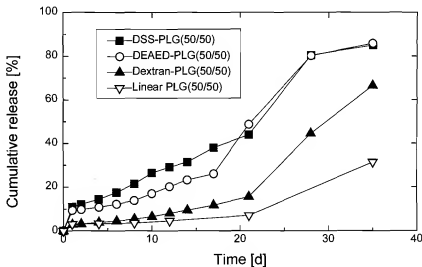


Figure 10 *In vitro* release of FITC-dextran (40 000) from the microspheres prepared from the graft and linear PLG(50/50)

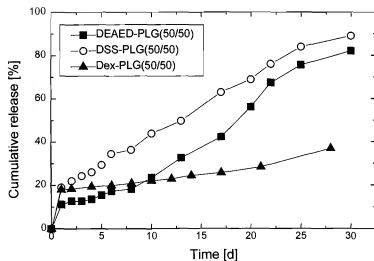


Figure 11 *In-vitro* release of BSA from the microspheres prepared from the graft PLG(50/50)

The *in-vitro* release profiles of BSA from the microspheres prepared from the graft PLG are similar to those of FITC-dextran as demonstrated in Figure 11. The initial drug release rate is slightly higher than FITC-dextran, after one day, a continuous release of BSA occurs for up to 30 d. The cumulative amount of BSA release within 30 d is ca. 80%. Again the charge effect of the backbone on the *in vitro* release behaviour becomes evident comparing DEAED-PLG(50/50) and DSS-PLG(50/50) to the uncharged DEX-PLG(50/50). In the latter case only ca. 30% of BSA is released over 30 d. No aggregation of BSA was observed by SDS-PAGE (data not shown), suggesting the internal environment in the matrix was more porous and hence less acidic than PLG.

The design of structure and composition of the graft PLG containing charged functionalities in the backbone yields biodegradable polyesters with beneficial properties for a continuous *in vitro* release of hydrophilic marker molecules from microspheres. These properties cannot be attained by blending or coencapsulation of excipients, as we have recently shown for rh-erythropoietin³⁸. Therefore, graft PLG may be of particular interest for parenteral delivery systems of proteins.

CONCLUSIONS

Biodegradable graft PLGs containing hydrophilic, charge-

modified dextrans as backbone are potential biomaterials for the controlled release of proteins and peptides. The mechanism of the degradation is influenced by the charged dextran. DSS-PLG shows a nonrandom hydrolysis of the chain which leads to a fast degradation of both weight-average and number-average molecular weight. DEAED-PLG shows a predominant hydrolysis of random chain scission. SEM micrographs and GPC data demonstrate that the erosion profiles of polymeric films are heterogeneous, which may change to a homogeneous degradation mechanism when degradation is accelerated by varying the LA/GA ratio. This leads to a faster degradation rate of the graft PLG, accompanied by a higher water content and porous structure.

The *in-vitro* release profiles of FITC dextran and BSA from the microspheres prepared from the graft PLG are almost continuous and allow the design of the protein delivery systems with 30 d release periods.

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Table 2 Preparation of the microspheres of the graft and linear PLG containing FITC-dextran and proteins

Polymers	LA/GA	Substance encapsulated	Drug loading (%)	Encapsulation (%)	Average size (μm)
DEAED-P- 50/50 LG		FITC-dex-tran	4.6	92	30-60
DEAED-P- 50/50 LG		BSA	4.7	94	30-60
DSS-PLG 50/50		FITC-dex-tran	4.3	86	30-60
DSS-PLG 50/50		BSA	4.3	86	30-60
Dex-PLG 50/50		FITC-dex-tran	4.5	90	30-60
Dex-PLG 50/50		BSA	4.4	88	30-60
Linear PLG 50/50		FITC-dex-tran	4.6	87	30-60

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